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**Carbohydrate particles contg. biologically active substances - stabilised
by crystallisation of the carbohydrate**

Patent Assignee: SCHRODER U (SCHR-I)

Inventor: SHRODER U

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AU 8317760	A	19840208				198417
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EP 113749	A	19840725	EP 83902308	A	19830704	198430
JP 59501213	W	19840712	JP 83502406	A	19830704	198434
FI 8400923	A	19840307				198450
DK 8400952	A	19840223				198502
ES 8505250	A	19850901				198602
CA 1222457	A	19870602				198726
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EP 113749	B	19880601				198822
DE 3376797	G	19880707				198828

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Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
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WO 8400294	A	E	17		
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Designated States (National): AU DK FI JP NO US

Designated States (Regional): AT BE CH DE FR GB LU NL SE

EP 113749	A	E			
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Designated States (Regional): AT BE CH DE FR GB LI LU NL SE

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Abstract (Basic): EP 113749 A

A process of producing a stable carbohydrate sphere or particle in the size range of 0.01 to 1,000 micro m, characterised in that a carbohydrate polymer is dissolved in one or more solvents having a dielectricity constant of more than 35 to a concentration within the range of 0.1 to 200% (weight/volume) to form a clear soln. whereupon the thus obtained hydrophilic carbohydrate polymer soln. is emulsified in a hydrophobic emulsion medium to form spherical droplets of the carbohydrate soln., whereafter the emulsion is stabilised by transferring it to a liquid capable of crystallising the carbohydrate polymer to a complex relatively insoluble in water.

(7pp)

Title Terms: CARBOHYDRATE; PARTICLE; CONTAIN; BIOLOGICAL; ACTIVE; SUBSTANCE ; STABILISED; CRYSTAL; CARBOHYDRATE

Derwent Class: A96; A97; B04; B07; C03; D16

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(21) International Application Number: PCT/SE83/00268 (22) International Filing Date: 4 July 1983 (04.07.83) (31) Priority Application Number: 8204244-1 (32) Priority Date: 9 July 1982 (09.07.82) (33) Priority Country: SE (71)(72) Applicant and Inventor: SCHRÖDER, Ulf (SE/SE); Fagottgränden 11 B, S-223 68 Lund (SE). (74) Agents: WALLIN, John et al.; Awapatent AB, Box 5117, S-200 71 Malmö (SE). (81) Designated States: AT (European patent), AU, BE (Eu- ropean patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (Euro- pean patent), JP, LU (European patent), NL (Euro- pean patent), NO, SE (European patent), US.		Published <i>With international search report.</i> <i>In English translation (filed in Swedish).</i>
(54) Title: A CRYSTALLISED CARBOHYDRATE MATRIX FOR BIOLOGICALLY ACTIVE SUBSTANCES, A PROCESS OF PREPARING SAID MATRIX, AND THE USE THEREOF (57) Abstract The invention shows that it is possible to produce a depot matrix for biologically active substances, consisting of carbohydrate microspheres, such that the carbohydrate polymers included in the microsphere are stabilised to a microsphere by crystallisation, which implies using non-covalent bonds, the substance enclosed retaining its biological activity.		

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A CRYSTALLISED CARBOHYDRATE MATRIX FOR BIOLOGICALLY
ACTIVE SUBSTANCES, A PROCESS OF PREPARING SAID
MATRIX, AND THE USE THEREOF

Background. Biologically active substances supplied to an organism are, in most cases, rapidly digested by the organism. In view hereof, the supply of substances must be repeated at regular intervals in order to establish a therapeutically active concentration within the organism. Such supply of biologically active substances to organisms is important int.al. in the fields of human and veterinary medicine, or in controlling different types of infestants (such as insects, fungi etc.) in agriculture and forestry.

In order to avoid the disadvantages of repeated administration, it is endeavoured to find matrices which, together with the substance, provide a depot effect, by which is meant that the substance in one way or another is adsorbed, coupled to or enclosed in a matrix from which it is then released, via different mechanisms, during a prolonged period of time. The great advantage of this type of administration is that the substance is supplied to the organism at a uniform rate; the peaks and valleys in concentration encountered with normal administration are avoided. By biologically active preparations are meant such preparations or substances as are capable of affecting organisms. Examples of such preparations are therapeutically active preparations, insecticides or herbicides, enzymes, antibodies, antigens, allergens, hormones, live or killed and whole or decomposed microorganisms or virus. As practical examples of the fields of use of the present invention, mention may be made of

1. Insulin: Patients suffering from diabetes must inject insulin at regular intervals or during meals in order to maintain



the blood sugar content at an acceptable level. Great advantages would be obtainable if a depot preparation of insulin could be administered in a simple manner, implying that the injection frequency could be reduced considerably;

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2. Vaccination: For the vaccination of humans, adjuvants cannot be used. However, it has been shown in literature that the immunogenic response will be far better if the body is subjected to long-time exposure of the antigen. For vaccination purposes, it is important that the depot matrix is not itself immunogenic, and that it can be excreted from the body. For example, vaccination tests conducted on human beings against bee allergens have shown that these allergens, dissolved in water and injected subcutaneously, are excreted within 4 hours.

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The production of depot matrices for different types of preparations is well-documented in literature, and some preparations are also commercially available.

The invention described in the present application uses polymers as depot matrix. Different types of polymers are described in literature. (Chem. Eng. Commun. (1980) 6, 1-48 or Int. J. Pharm. (1980) 7, 1-18). Among the desirable properties of such a polymer preparation are the following:

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1. The polymer should in itself be chemically inert in biological systems.
2. The polymer should be biologically well-characterised.
3. The polymer should be non-toxic and non-immunogenic.
4. The polymer should be excretable from the body via normal routes.
5. The polymer preparation should be readily administrable.



6. The polymer preparation should be capable of releasing a biologically active substance, and the release rate of the active substance should be readily controllable.

5 7. The polymer preparation should be able to enclose and release substances of different molecular weights.

Existing polymer systems described in the above-mentioned summary reviews are all of the type covalently cross-linked polymers in which the covalent cross-linkage in some cases is unstable in biological systems, and in which the biologically active substance is covalently bonded to the polymer. This instability causes degradation of the polymer preparation, whereby the preparations are released.

15 A different type of release is obtained if crystalline substances (such as crystallised proteins) are enclosed in the covalently cross-linked polymer preparation. By utilising a varying degree of porosity in the polymer preparation, varying release times are obtained.

20 In general it may be said that it is endeavoured, in developing polymer preparations for administration of biologically active substances, to use polymers which are as "pure" as possible. One such "pure" polymer class is represented by the carbohydrates. Presentday methods of preparing carbohydrate matrices comprise a covalent coupling of the polymer chains included (GB 924054) or heat treatment (SE 4024/69) in order to obtain stable matrices.

30 Description of the invention

The invention is based upon a novel technique for stabilising carbohydrate polymers according to the appended claims. This technique involves emulsifying a solution of the polymer in a hydrophobic emulsifying medium, whereby spherical droplets of the soluble carbohydrate polymer are obtained in the emulsifying medium. To stabilise the sphere, the said emulsion is then poured into



a liquid capable of crystallising the carbohydrate polymer to a complex relatively insoluble in water.

Crystallisation implies that the type of bonds holding the carbohydrate polymers together in a micro-
5 sphere is chemically characterised as non-covalent of the type hydrogen bonds, ion bonds or van der Waals forces, the majority consisting of hydrogen bonds.

The resulting polymer matrix has such characteristics that it can retain biologically active substances
10 in the non-covalently cross-linked polymeric lattice, the biologically active substance being released concurrently with the slow redissolution of the crystallised carbohydrate matrix.

The simplest way of incorporating the biologically
15 active substance is to admix it to the dissolved carbohydrate polymer before this is mixed with the emulsifying medium.

The matrix material in the production of these spheres or particles consists of carbohydrate polymers.
20 The technique described in the present invention makes it possible to use the carbohydrates dextran, pullullan, glucogen, starch, agarose, cellulose, alginate, chitosan or carrageenan, and different derivatives thereof.

In some cases, also the carbohydrate per se may
25 be of interest as a biologically active substance. One example hereof is the carbohydrate heparin which is used therapeutically as an anticoagulant.

As regards the molecular weight of the carbohydrates, the technique has been shown to function within
30 a very large range. For instance, crystallised carbohydrate spheres have been produced from glucose, sucrose and maltose. Dextrans and dextrans having an average molecular weight from about 800 up to several millions have also been shown to function. The upper limit of
35 the molecular weight in the preparation of crystallised carbohydrate spheres according to the present invention is limited only by the solubility of the respective carbohydrates in their solvents.



The modification of these carbohydrate polymers to different types of derivatives can be carried out in such a manner that the ability of the carbohydrates to adsorb for example hydrophobic or charged substances, is changed. Examples of well-documented hydrophobic substituents are cholesterol, DEAE, or Ciba chrome blue which all can be covalently coupled in simple manner to the carbohydrate polymer. Correspondingly, charged groups of the type $-SO_4$ or $-NH_2$ can be covalently coupled to the carbohydrate matrix for adsorption of charged substances. The above-mentioned substituents are commercially available, coupled to the carbohydrate polymer dextran, from Pharmacia AB at Uppsala.

Of the above-mentioned carbohydrate polymers, the polymers dextran, starch, glucogen, or pullullan are preferred on grounds previously described (see points 1-7, on pp. 2-3). The main reason is that these polymers are extremely well characterised in biological systems. Of the above-mentioned polymers, however, dextran must be put in the first place as an example of a non-enzymatically (in tissue) degradable matrix for biologically active substances. Where starch, glucogen or pullullan is concerned, the alpha-amylase concentration determines the time of degradation and thus also the release time of the biologically active substances. In the case of dextran, it is only the physical parameters, such as the pH or ionic strength, which redissolve the crystallised matrix and thus control the release.

The disadvantage of an enzymatically controlled release is that, in the case of for example alpha-amylase, the concentration thereof may vary within large ranges in body fluids for different diseases. In other cases, the enzyme concentrations may vary between patients or in the same patient at different times of the day and thus affect the release time of the biologically active substance.

The present invention describes how one prepares



crystallised carbohydrate spheres with enclosed biologically active substances. The method is characterised in that the carbohydrate polymer is dissolved in a solvent having a high dielectricity constant to a concentration lying within the range 0.1-200% (weight/volume). By high dielectricity content is here meant a solvent or combinations of solvents having a dielectricity constant of more than about 35. Useful such solvents are, inter alia, dimethyl formamide, ethylene glycol, dimethyl sulphoxide, water and formamide, or mixtures thereof. The biologically active substance is added to this solution. The resulting mixture of dissolved carbohydrate polymer and biologically active substance is then emulsified in an emulsion system comprising the said solution and an emulsion medium consisting of a liquid which is immiscible with said solution and which has the further characteristic of contributing to the formation of droplets of the carbohydrate solution in the emulsion medium.

As examples of useful emulsion media mention may be made of vegetable oils, preferably rapeseed or maize oil. Other useful hydrophobic emulsion media include paraffin oils or silicone oils. Another type of emulsion medium includes organic solvents in which one or more emulsifiers have been dissolved. Useful such organic solvents include, inter alia, xylene, toluene, ethyl benzene, diethyl benzene, propyl benzene, ethylene chloride and other similar solvents, as well as mixtures thereof.

The technique of using emulsion media in combination with different emulsifiers in order to obtain varying diameters in the preparation of microspheres is well documented in literature and will not be described in detail in the present context.

To emulsify the emulsion, use is made of a sonicator or high-pressure homogeniser. The resulting emulsion in which the carbohydrate solution is emulsified



in the form of droplets, is stabilised by transferring it to a liquid capable of crystallising the carbohydrate polymer, whereby the biologically active substance is enclosed. Useful such liquids are ethanol, methanol or acetone, although the latter is preferred. After crystallisation, the resulting matrix is further washed with acetone, whereupon drying is effected simplest by rotational evaporation or in a warming cupboard.

It is important that the enclosed substances retain their biological activity also after release from the matrix.

In this respect, the present invention shows that hormonal proteins of the type insulin and interferon, enzymes such as plasmin and beta-galactosidase as well as monoclonal antibodies retain their biological activity after enclosure and subsequent release from the matrix.

In some cases, it is not possible to enclose substances within the matrix. One then has the possibility of covalently coupling the substance to the matrix, in which case it is important that the technique of covalent coupling does not imply any appreciable degree of cross-linking of the matrix because such cross-linking would completely annihilate the release mechanisms upon dissolution of the carbohydrate matrix, as has previously been discussed.

Such a type of covalent coupling is obtained if use is made of tresyl chloride as the activating substance because this coupling technique does not result in cross-linking of the carbohydrate matrix (Biochem. Biophys. Res. Comm. (1981) 102, 449-457).

The following Examples are not to be regarded as restrictive, but rather as illustrative of the main features of this invention.

EXAMPLE 1

1 gram of a 50% (weight/volume) aqueous solution of dextran, having a molecular weight of 40,000, was



mixed with 100 μ l of an ovalbumin solution containing 100 mg of ovalbumin/ml of water. 5 μ l of 125 I-labeled ovalbumin had previously been added to the latter solution.

5 The dextran-ovalbumin solution was suspended in 25 ml of vegetable oil in a 100 ml beaker and cooled to $+4^{\circ}\text{C}$. The mixture was emulsified by ultrasonics for 1 minute, whereupon the emulsion was poured into 200 ml of acetone in which the emulsifier Tween 80 had been
10 dissolved to a concentration of 0.1% (weight/volume). While the emulsion was being carefully poured into the acetone solution, it was stirred at about 1,000 rpm. The resulting dextran spheres which had been stabilised by crystallisation and contained ovalbumin enclosed
15 therein were washed 4 times more with the said acetone solution, whereupon they were air-dried.

Normally, such a test gives a recovery of about 250 mg of spheres in which 60-70% of ovalbumin added have been enclosed in the carbohydrate matrix. The size
20 of the spheres prepared by this technique lies between 0.01 and 10 μm . By varying the composition of the emulsion medium, spheres having a diameter of up to 1 mm are readily produced.

EXAMPLE 2

25 The same as in Example 1, but with the difference that the dextran was crystallised in methanol or ethanol, instead of acetone.

EXAMPLE 3

30 The same as in Example 1, but with the difference that 0.2 g of starch was dissolved in water, and that the starch solution was emulsified in toluene containing the emulsifier Gafac PE-510 (5% weight/volume), instead of dextran and oil.

EXAMPLE 4

35 The same as in Example 1, but with the difference that 1 g of 0.2% agarose or carageenan at a temperature of $+40^{\circ}\text{C}$ was used instead of dextran.



EXAMPLE 5

The same as in Example 1, but with the difference that 1 g alginate was used instead of dextran and emulsified by means of a 0.1% admixture of the emulsifier
5 Gafac RM-410 into the oil instead of pure oil.

EXAMPLE 6

The same as in Example 1, but with the difference that 1 g of 1% chitosan dissolved at pH 5 was used instead of dextran.

10 EXAMPLE 7

The same as in Example 1, but with the difference that 3 g of cellulose were dissolved in 150 g of N-ethyl pyridine chloride and 75 g of dimethyl formamide, 1 g of this solution being used instead of dextran.

15 EXAMPLE 8

The same as in Example 1, but with the difference that a 200% (weight/volume) aqueous solution of sucrose was used instead of dextran.

EXAMPLE 9

20 The same as in Example 1, but with the difference that a 30% (weight/volume) aqueous solution of glycogen was used instead of dextran.

EXAMPLE 10

25 The same as in Example 1, but with the difference that ovalbumin was not used, and that the per se biologically active carbohydrate polymer heparin was dissolved to a concentration of 50% (weight/volume) in water and used instead of dextran for the production of crystallised spheres.

30 EXAMPLE 11

100 mg of dried spheres according to Example 1 were slurried in 10 ml of PBS at pH 7.2. At different times it was investigated how much of the enclosed ovalbumin had been released from the spheres by filtrating
35 the mixture through a filter having a molecular "cut-off" of 100,000.

With the above-mentioned spheres, a half-life of released ovalbumin of about 12 days is obtained.



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By varying the concentration or the molecular weight of the dextran polymer, the half-lives can be varied in simple manner. By increasing the concentration or using a higher molecular weight of the polymer, a longer half-life is obtained.

EXAMPLE 12

As in Example 1, it is possible to use substituted dextrans in the production of the spheres. To investigate the documented adjuvant effect of DEAE and SO_4 -substituted dextrans, these were mixed with unsubstituted dextran at the ratios 0%, 33%, 66% and 100%. Such spheres with enclosed ovalbumin were then injected subcutaneously in mice, whereupon IgG and IgM antibodies against ovalbumin were determined by means of a so-called micro-titer-ELISA-method.

It proved that the immunological response was directly correlated to the amount of adjuvant, and that the antibody titer of the mice that were given ovalbumin in unsubstituted dextran spheres was significantly higher as compared with the mice injected with the ovalbumin merely dissolved in water.

EXAMPLE 13

To investigate whether a biologically active protein retains its activity, insulin was enclosed in dextran spheres made from a 20% (weight/volume) dextran solution. The biological activity was then evaluated in that the insulin released from the spheres was shown to retain its capacity for in vitro stimulation of fat cells to produce fatty acids. At a release test conducted on enclosed insulin, tested in the same manner as in Example 2, but with ^{125}I insulin, there was obtained from spheres prepared from a 35% solution of dextran having a molecular weight of 500,000, a half-life of about 6 days.

EXAMPLE 14

As in Example 10, interferon was enclosed in dextran spheres, whereupon the antiviral effect of the interferon was determined in a so-called plaque-assay.



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About 35% of the antiviral effect of the interferon supplied could be detected.

EXAMPLE 15

5 As in Example 10, the enzyme plasmin was enclosed in dextran spheres, whereupon its enzymatic activity was determined by means of the substrate H-D-Val-Leu-Lys-pNA after release from the spheres. The dried spheres contained about 1% of plasmin with a recovery of the biological activity of about 75%.

10 EXAMPLE 16

As in Example 10, the enzyme beta-galactosidase was enclosed in dextran spheres, whereupon the enzymatic activity was determined after release from the matrix by means of the substrate ONPG.

15 The dried spheres contained about 5% of the enzyme with a recovery of biological activity of 70%.

EXAMPLE 17

20 As in Example 10, a monoclonal antibody directed against the protein PHA was enclosed in the spheres, whereupon its binding activity was determined in a so-called sandwich-ELISA after release from the matrix. Recovery of the biological activity was 65%.

EXAMPLE 18

25 As in Example 10, 125-I labeled biosynthetic growth hormone was enclosed, although in this instance an 80% solution of a dextrin (Awedex W90, Stadex AB, Malmö) was employed.

Recovery of radioactivity was 100%.

EXAMPLE 19

30 A 200% (weight/volume) solution of maltose was prepared, whereupon the pharmaceuticals metotrexate and vincristine (³H-labeled), respectively, were enclosed in spheres by the same technique as in Example 1.

35 Recovery of metotrexate was 82% and for vincristine 22%.



EXAMPLE 20

As in Example 19, metotrexate and vincristine were enclosed in the carbohydrates dextran T1 and glucose.

Recovery for metotrexate was for both carbohydrates 65%, and for vincristine a recovery of 40% was obtained.

EXAMPLE 21

As in Example 1, spheres were produced, but with the difference that 1 g of 30% (weight/volume) dextran T500 was used as carbohydrate, and that 250 μ l of albumin (100 mg/ml) were added for enclosure.

In this manner, 65% of added protein were enclosed, which corresponds to 40% of the dry weight of the spheres.

EXAMPLE 22

In present day treatment of bee allergies on human beings, successively higher doses of an aqueous solution of the allergen are injected.

To test whether the immunological response is changed if a depot preparation of bee allergens is used, bee allergen was enclosed in dextran spheres according to Example 1 and injected subcutaneously in mice whereupon the immunological response with respect to IgG was determined at different times. For comparison, bee allergen and pure water and bee allergen suspended in Freund's complete adjuvant were injected. The antibody content in the group that had been given bee allergen dissolved in water, showed a slight rise after 1 week and then receded to undetectable contents. On comparing the depot preparation to Freund's adjuvant, it was found that the antibody content rose more quickly when the bee allergens were enclosed in the depot matrix. After 10 weeks, the IgG contents in serum were still rising for both groups, which shows that the depot preparation is highly efficient when it is desired to obtain high contents of antibodies.

EXAMPLE 23

1 gram of aqueous solution of dextran having a molecular weight of 10,000 was mixed with 50 μ l of the



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low-molecular pharmaceutical metotrexate. The total amount added was 5 mg which, besides, was 3-H-labeled. The mixture was processed according to Example 1 and the result was that 92% of metotrexate added were enclosed in the dried spheres.

EXAMPLE 24

To investigate the release from a matrix degradable by enzymes, the following test was carried out.

100 mg of spheres produced from starch dissolved in formamide according to Example 1, were activated with tresyl chloride, whereupon 125-I myoglobin was coupled to the spheres. After careful washing to remove adsorbed myoglobin, alpha-amylase was added in a concentration that was 100 times higher than in normal human serum. Within two hours, about 25% of coupled myoglobin had been released from the matrix. By further increasing the amount of alpha-amylase 10 times during the next 24 hours, a total release of about 45% of coupled myoglobin was obtained. During the last 24 hours, the alpha-amylase concentration in the test was about 20,000 times higher than in normal human serum.



CLAIMS

1. A carbohydrate sphere or particle in the size range 0.01-1,000 μm with enclosed, adsorbed or covalently bonded biologically active substances in a dry-weight percentage ratio of enclosed substance to matrix within the range 0.001-50, characterised in that the carbohydrate sphere or the particle is stabilised by crystallisation.

2. The sphere or particle as claimed in claim 1, characterised in that it has an average diameter within the range 0.01-1,000 μm , preferably below 1 μm .

3. The sphere or particle as claimed in claims 1-2, characterised in that the carbohydrate is selected from the group consisting of dextran, starch and the derivatives thereof.

4. The sphere or particle as claimed in claims 1-2, characterised in that the carbohydrate is selected from the group consisting of alginate, chitosan, agarose, carrageenan, cellulose, glucogen, pullulan and the derivatives thereof.

5. A process of producing the sphere or particle as claimed in claims 1-4, characterised in that, during production, the carbohydrate is dissolved in one or more solvents to form a clear solution, whereupon the hydrophilic carbohydrate polymer solution is emulsified in a hydrophobic phase to form spherical droplets which are stabilised by crystallisation.

6. A process of producing the sphere or particle as claimed in claim 5, characterised in that the crystallising medium used is acetone, ethanol or methanol, although acetone is preferred.

7. Use of the spheres or particles as claimed in claims 1-6, together with antigen.



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8. Use of the spheres or particles as claimed in claims 1-6, together with insulin.

9. Use of the spheres or particles as claimed in claims 1-6, together with allergens.



INTERNATIONAL SEARCH REPORT

International Application No PCT/SE83/00268

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC 3		
A 61 K 9/14		
II. FIELDS SEARCHED		
Minimum Documentation Searched *		
Classification System	Classification Symbols	
IPC 3	A 61 K 9/00, 14, 16, 18, 34, 52, 35/00, 39/385	
National Cl	30h:9/02	
US Cl	424:14, 19	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *		
SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT 14		
Cat	Citation of Document, 14 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
A	EP, A, 0 021 009 (BASF AG) 7 January 1981	1 - 9
A	EP, A, 1 021 230 (PHARMACIA AB) 7 January 1981	1 - 9
A	Patent Abstracts of Japan, abstract of JP 52-136911, published 1977-11-16	1 - 9
A	Patent Abstracts of Japan, abstract of JP 53-131109, published 1980-04-28	1 - 9
A	Patent Abstracts of Japan, abstract of JP 55-47615, published 1980-04-04	1 - 9
<p>* Special categories of cited documents: 19</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search *	Date of Mailing of this International Search Report *	
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